Characterisation of humic substances extracted from soil treated with charcoal (biochar)

Novotny EH[a*], Auccaise R[a], Lima LB[bc], Madari BE[b]
[a] Embrapa Soils, Rua Jardim Botânico, 1024, CEP 22460-000, Rio de Janeiro-RJ, Brazil, [b] Embrapa Rice and Beans, Caixa Postal 179, CEP 75375-000, Santo Antônio de Goiás – GO, Brazil, [c] Federal University of Goiás, Caixa Postal 131, CEP 74690-900, Goiânia – GO.
* E-mail: etelvino@cnps.embrapa.br
Keywords biochar; pyrogenic carbon; humic acids; humin; NMR; chemometry

Abstract The high fertility and resilience of *Terras Pretas de Índios* are attributed to the high contents of polycondensed aromatic structures with carboxylic functionality originated from the natural alterations of carbonised biomass added to the soil by the pre-Columbian indigenous populations. There are several proposals to reproduce the positive characteristics of these special soils, including the use of charred biomass (biochar), however the time necessary to modify this material in the peculiar organic matter of *Terras Pretas de Índios* is still undetermined. The ^{13}C NMR analysis of the humic acids extracted at pH 10.6 from soils treated with fines of charcoal indicates that after three years a small portion of the added charcoal is already partially oxidised to a compound similar to the ones found in *Terras Pretas de Índios*, however the large fraction still looks like unaltered charcoal. Additionally, the proposed method of selective extraction of the characteristic humic fraction from *Terras Pretas de Índios*, combined with multivariate data analysis tools, can be used as a method to evaluate the proposals to reproduce these special soils.

Introduction

Terras Pretas de Índios are anthropogenic soils found in the Amazon that have high carbon content, high fertility and high resilience. These characteristics are due to the pyrogenic character of their organic matter. Based on several investigations of the organic matter from *Terras Pretas de Índios*, an efficient model of organic material has been proposed, that can provide sustainability to agricultural production and result in carbon sequestration. This model involves a compound of polycondensed aromatic structures with carboxylic functionality (Novotny et al., 2009).

Charred materials have condensed aromatic groups that guarantee their recalcitrance in the environment (half-life ranging from centuries to millennia), being so an efficient material for carbon sequestration. Its application in the soil is followed by biological and chemical transformations through which carboxyl groups are directly connected to the recalcitrant aromatic structures. After those transformations, the final compound attributes high fertility to the soil. This is what makes *Terras Pretas de Índios* soils so fertile, differentiating them from other soils in the Amazon (Glaser et al., 2001; Novotny et al., 2009).

However, this transformation process in nature occurs in a time lapse still undetermined. Seeking to following the structural changes in charcoal fines (< 2 mm), produced by conventional carbonization, and their incorporation into the humic fractions of the soil organic matter, a field experiment with different doses of charcoal was installed in 2008, and the humic acids and humin fractions were analysed by solid-state ^{13}C-NMR spectroscopy.

Materials and methods

The soil samples were collected from a field experiment (four randomised block design) installed in a Dystric Plinthosol, textural class sandy loam,in Nova Xavantina – MT, Brazil (14º 41´ 48" S and 52º 20´ 55" W and 310 m amsl). The treatments were 0; 16 and 32 Mg ha^{-1} of *Eucalyptus* sp. charcoal fines, incorporated with rotary hoe. The sampling was done two years after the experiment installation. For the humic acids extraction, a modified IHSS procedure was employed, this modification consists of an exhaustive and sequential extraction of the humic acids with an aqueous solution with pH adjusted at 10.6 and thereafter at pH 12.6 (Song et al., 2008). The first extract (pH 10.6) intends to preferably extract the carboxylated aryl components while the second step (pH 12.6) intend to extract the remain humic and fulvic acids (ordinary soluble humic substances). This approach works very well in pyrogenic carbon rich soils, such as *Terras Pretas de Índios*. For the humic acids isolation, both extracts (pH 10.6 and 12.6) were acidified to pH~1 with 6M HCl and separated by centrifugation. The residual humin was recovered using a 10% HF plus 0.1M HCl solution. The humic acids (pH 10.6 and 12.6) and the humin were dialysed and freeze dried. Variable-amplitude cross-polarization (VACP) solid-state ^{13}C NMR experiments were carried out using a 500 MHz Varian spectrometer at ^{13}C and ^{1}H frequencies of 125 and 500 MHz, respectively. Magic-angle spinning (MAS) at 15 kHz was employed. Typical cross-polarisation times of 1 ms, acquisition times of 16.4 ms, and recycle delays of 500 ms were used. High-power Two-Pulse Phase-Modulation (TPPM) proton decoupling of 70 kHz was applied. To aid in the analyses of the results, the Multivariate Curve Resolution (MCR) procedure was carried out using the software "The Unscrambler X, v. 10.2" (CAMO Software AS), to facilitate the modelling, additional spectra from humic acids extracted of *Terras Pretas de Índios* and a model functionalised charcoal were included in the MCR analysis. For the MANOVA analysis, both humic acid fractions from 0 and 16 Mg ha^{-1} were analysed in duplicate (two experimental blocks). Due to the observation dependence (extract at pH 10.6 and 12.6), and consequently an expected residual dependence, both extracts were treated as individual response variables in a Factorial (charcoal doses x blocks) MANOVA approach, instead a unique variable in a conventional transverse Factorial ANOVA design (charcoal doses x pH extractor x blocks).

Results and discussion

The MCR indicates that the humic substances can be modelled by means of a three-component mixture (Fig. 1a): one component of ordinary tropical humic substances with signals of alky, N-alkyl, methoxyl, carbohydrates, aryl, O-aryl, aliphatic carboxyl and amide. These chemical groups indicate the presence of plant material in different humification stages; one with a polycondensed and carboxylated aromatic structure (oxidised charcoal); and the last one, typical of unaltered charcoal. The carboxylic region in the spectrum of the ordinary humic substances component is mainly from aliphatic carboxyl and amide groups (173 ppm) whereas, when this group is linked directly to the aromatic structures, its signal shits to 168 ppm (vertical dashed line in the Fig. 1a).

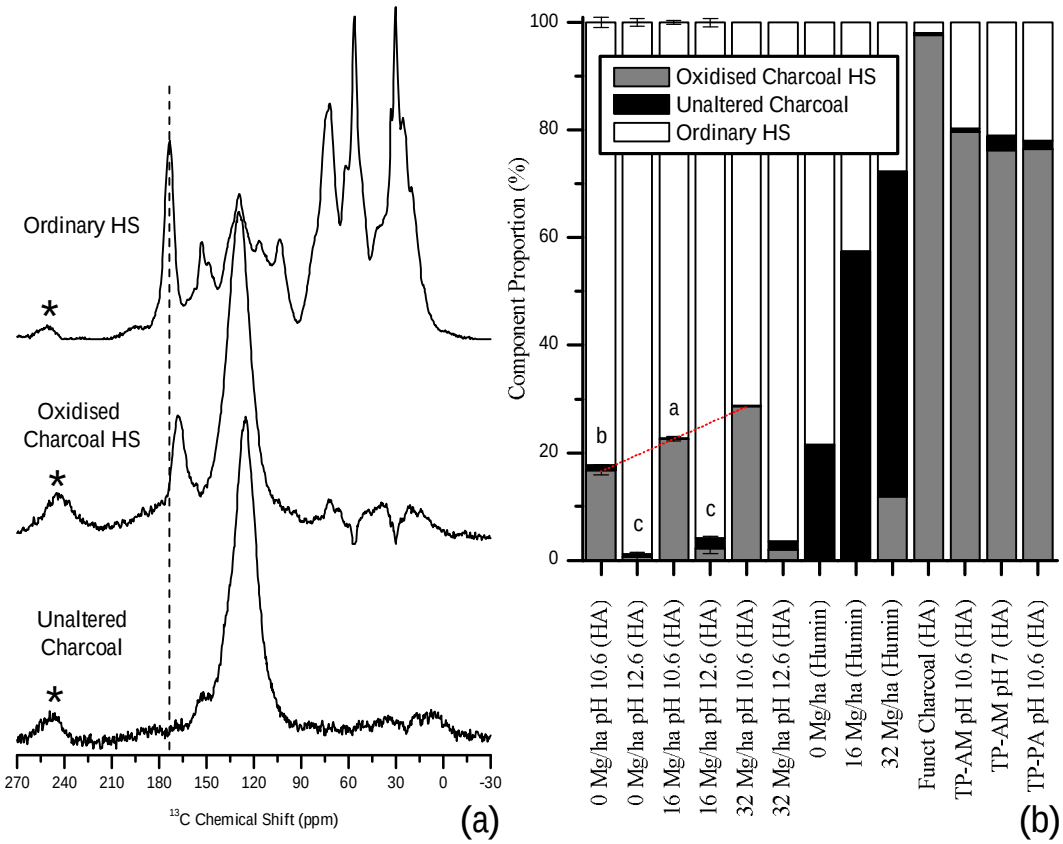

Fig. 1 Results of multivariate curve resolution (MCR) analysis. (a) Estimated spectra; (b) Estimated concentrations. * spinning sidebands; HS: humic substances; HA: humic acids; TP: *Terra Preta de Índio* samples; AM: samples from Amazonas state; PA: sample from Pará State; pH #: pH of the extraction solution. Bars are the standard error of the means of two or three experimental blocks. Gray bars with the same letters do not differ at 5% significant level. The dotted red line is the linear fitting of charcoal doses and oxidised charcoal content.

The extracts from pH 10.6 were richer in oxidised charcoal than the ones from pH 12.6 (Fig. 1b), confirming its preferential extraction at pH 10.6, besides, the control treatment (0 Mg ha^{-1}) already presents significative amount of oxidised charcoal, probably from native charcoal originated by natural and induced fires, common in the Brazilian savannah region. It is possible to fit a linear relationship (R^2 = 0.99, insert in Fig. 1b) between charcoal doses and oxidised charcoal component, proving that just after two years, a small proportion of fresh charcoal is already oxidised to polycondensed aromatic structures with carboxylic functionality, similar to the ones found in *Terras Pretas de Índio*. However, the large proportion of the applied charcoal is still found as unaltered charcoal in the humin fraction (Fig. 1b).

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